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Research Article

New species and records of Botryosphaeriales (Dothideomycetes) associated with tree dieback in Beijing, China

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Abstract

Botryosphaeriales species are important pathogens that have worldwide distribution. In this study, 23 Botryosphaeriales strains were isolated from 13 host species during a dieback disease survey in Beijing, China. Based on morphological and phylogenetic analyses, six Botryosphaeriales species were identified, including two new species named *Dothiorella hortiarborum* **sp. nov.** and *Phaeobotryon fraxini* **sp. nov.**, and four new host records: *Aplosporella ginkgonis* from *Cotinus coggygria* var. *cinereus*, *A. javeedii* from *Acer miyabei*, *Acer truncatum*, *Forsythia suspensa*, *Lagerstroemia indica*, *Sambucus williamsii*, *Syringa vulgaris*, *Ulmus pumila*, *Xanthoceras sorbifolium*, *A. yanqingensis* from *Acer truncatum*, and *Do. acericola* from *Forsythia suspensa*, *Ginkgo biloba*, and *Syringa oblata*. This study enriches the species diversity associated with tree dieback in Beijing, China, and contributes to the further study of the taxonomy of this order.

Key words: *Dothiorella*, morphology, *Phaeobotryon*, phylogeny, taxonomy

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Introduction

Botryosphaeriales species are important plant pathogens commonly found on the trunks and branches of woody plants (Phillips et al. 2013; Lawrence et al. 2017; Zhu et al. 2018; Zhang et al. 2021). They are associated with branch canker, dieback, decline, and death, with consequences for the ecological and economic value of the forest (Slippers and Wingfield 2007; Phillips et al. 2013; Mohali-Castillo 2023). Botryosphaeriales species occur on a wide range of hosts, in the form of endophytes on woody plants and herbs, lichens, and even seaweed leaves in marine environments, suggesting that they have great potential for research value (Yang et al. 2017; Akinsanmi et al. 2019; Zhang et al. 2021; Mohali-Castillo 2023; Rathnayaka et al. 2023).

Phylogenetic analyses of DNA sequence data have an enormous influence on the systematics and taxonomy of the order Botryosphaeriales, including redefining families and genera and identifying new species (Phillips et al. 2019; Mohali-Castillo 2023). Schoch et al. (2006) combined SSU, LSU, *tef1-α*, and *rpb2* to first propose the order Botryosphaeriales, which contains only a single family of Botryosphaeriaceae. Minnis et al. (2012) supplemented the DNA sequence data of Planistromellaceae with phylogenetic analyses combining

SSU, ITS, LSU, and *rpb1*, which introduced the family into the Botryosphaeriales. Wikee et al. (2013) reintroduced the Phyllostictaceae, grouped under Botryosphaeriales, to accommodate *Phyllosticta* using intronic genes (ITS, *act*, and *tef1-α*) and highly conserved coding regions of genes (LSU and GPDH). Slippers et al. (2013) added three new families, Aplosporellaceae (*Aplosporella* and *Bagnisiella*), Melanopsaceae (*Melanops*), and Saccharataceae (*Saccharata*), to Botryosphaeriales based on DNA sequence data of six loci (SSU, LSU, ITS, *tef1-α*, *tub2*, and mtSSU). Wyka and Broders (2016) introduced Septorioideaceae based on morphological and molecular evidence. Yang et al. (2017) mentioned that the LSU-*rpb2* combination could effectively classify taxa at the family and genus levels, and *rpb2* in combination with ITS, *tef1-α*, and *tub2* added additional resolution for species delimitation. For this reason, they combined the five fragments ITS, *tef1-α*, *tub2*, LSU, and *rpb2* to propose two new families, Endomelanconiopsisaceae and Pseudofusicoccumaceae. Therefore, Botryosphaeriales contained a total of nine families. However, Phillips et al. (2019) reassessed the families of Botryosphaeriales in terms of morphology of the sexual morphs and phylogenetic relationships of ITS and LSU sequence data, ultimately concluding that the order contained only six families (Aplosporellaceae, Botryosphaeriaceae, Melanopsaceae, Phyllostictaceae, Planistromellaceae, and Saccharataceae), with Endomelanconiopsisaceae, Pseudofusicoccumaceae, and Septorioideaceae as synonyms of existing families. Up to date, six families and 32 genera are accepted in Botryosphaeriales (https://www.outlineoffungi.org/). Of these, Botryosphaeriaceae is rich in species diversity, high in pathogenicity, and widely distributed.

Botryosphaeriaceae was first established by Theissen and Sydow (1918), containing three genera: *Botryosphaeria*, *Dibotryon*, and *Phaeobotryon*. Morphologically, Botryosphaericeae species are distinctive from other families in Botryosphaeriales by their large, ovoid to oblong, usually hyaline, aseptate ascospores (Phillips et al. 2013). Liu et al. (2012) assumed that ascospores could become pigmented and septate with age. Conidia in the asexual state of Botryosphaericeae are diverse in morphological characteristics (Phillips et al. 2005). Phylogenetically, however, there is a random distribution of hyaline or colored conidia or ascospores in the phylogenetic tree of Botryosphaericeae (Slippers et al. 2013). Therefore, accurate identification of species in the family by a single circumscription is not suitable. Currently, 22 genera and more than 200 species are contained within the family (https://www.outlineoffungi.org/). Recently, many new species have been introduced in the Botryosphaeriaceae, especially in the genera *Dothiorella* and *Phaeobotryon* (Jia et al. 2023; Li et al. 2023; Lin et al. 2023a; Wu et al. 2023).

Saccardo (1880) first established *Dothiorella* and designated *Do. pyrenophora* as the type species. Up to now, some scholars have made systematic revisions of *Dothiorella* to establish a more stable phylogenetic relationship (Dissanayake et al. 2016; Dissanayake et al. 2020; Zhang et al. 2021). The distinctive features of the genera are that the conidia are colored in the early stages of development, and with 1-septate, the sexual form of ascospores is brown and septate (Senanayake et al. 2023). The type species of the genus *Phaeobotryon* is *P. cercidis*, which is characterized by 2-septate brown ascospores with conical apiculate-elliptic to oblong or obovoid shapes at both ends and hyaline or brown conidia (Phillips et al. 2013; Fan et al. 2015b; Zhu et al. 2018; Pan et al. 2019).

In recent years, multiple studies have revealed that new species of Botryosphaeriales infest branches and trunks. Pan et al. (2019) found that *Phaeobotryon rhois* and *Diplodia quercicola* were detrimental to *Rhus typhina* and *Quercus variabilis* separately in Yudu Mountain, Beijing. *Aplosporella yanqingensis* and *Dothiorella baihuashan* are mainly recorded on Pinaceae or Cupressaceae (Lin et al. 2023a). *Lasiodiplodia regiae* caused the canker and dieback of apple trees (Wang et al. 2023). These studies suggest that Botryosphaeriales is rich in species diversity and has the potential to continue to be explored for new species. During the investigation of plant pathogens in Beijing, a higher number of diseased plant branches caused by Botryosphaeriales fungi were found. This study used phylogenetic analysis and morphological comparisons to describe new species and new host records, enriching the fungal taxa within Botryosphaeriales.

Materials and method

Sample collection and fungal isolation

A survey on dieback diseases was conducted from March to November 2023 in the Tongzhou District of Beijing, China. A total of thirteen tree species were examined, namely *Acer miyabei*, *A. truncatum*, *Cotinus coggygria* var. *cinereus*, *Forsythia suspensa*, *Fraxinus chinensis*, *Ginkgo biloba*, *Lagerstroemia indica*, *Sambucus williamsii*, *Styphnolobium japonicum*, *Syringa oblata*, *Syringa vulgaris*, *Ulmus pumila*, and *Xanthoceras sorbifolium*. Twenty specimens showing typical dieback symptoms (Fig. 1) with typical conidiomata and/or ascomata were collected. All samples were placed in paper bags and transported to the laboratory. Specimens with typical conidiomata pycnidial were selected for isolation. Removing the spore mass from conidiomata and generating single spore colonies or plating superficially sterilized diseased tissue on potato dextrose agar plates (PDA; containing 200 g potatoes, 20 g dextrose, and 20 g agar per liter) and incubating Petri dishes at 25 °C in the dark for 2–3 d. When colonies just formed, they transferred to fresh PDA Petri dishes (Crous et al. 2019). All specimens were deposited at the Museum of Beijing Forestry University (BJFC), and all cultures were preserved at the China Forestry Culture Collection Center (CFCC).

Morphological observation

Cultures were incubated on PDA at 25 °C in a 12-h day/night regime (Crous et al. 2019). After 14 days, the colonies were measured, and characteristics based on the color, shape, and sparseness of the aerial mycelium of the pathogen colonies were observed and recorded. Conidiomata were manually sectioned with a double-edged razor blade. Observations were conducted using a Leica DM 2,500 dissecting microscope (Wetzlar, Germany) and a Nikon Eclipse 80i compound microscope, equipped with differential interference contrast (DIC) illumination. Images were captured using a Nis DS-Ri2 camera with the Nikon Nis-Elements F4.30.01 software. Conidial length was measured from the base of the basal cell to the base of the apical appendage, while conidial width was measured at its widest point. A randomized selection of conidia was used for measurement (n = 50).

Figure 1. Disease symptoms associated with Botryosphaeriales species collected from Tongzhou District, Beijing, China **A** *Xanthoceras sorbifolium* **B** *Fraxinus chinensis* **C** *Lagerstroemia indica* **D** *Sambucus williamsii* **E** *Styphnolobium japonicum* **F** *Forsythia suspensa*.

DNA extraction, PCR amplification, and sequencing

Genetic DNA was extracted using the cetyltrime-thylammonium bromide (CTAB) method when the mycelium was well spread on the PDA. DNA samples were stored at -20 °C. The PCR reaction primers (forward and reverse) and amplification conditions are detailed in Table 1. Polymerase chain reaction (PCR) amplification was run on a PTC-200 Thermal Cycler amplifier from Bio-Rad, USA. The PCR amplification systems were all 20 µL, including 10 µL of Mix (Promega), 7 µL of double deionized water, 1 µL each of pre- and post-primers, and 1 µL of DNA template. PCR products were assayed by electrophoresis on 2% agarose gels. Amplified PCR products were sent to a commercial sequencing provider (Tsingke Biotechnology Co. Ltd., Beijing, China).

Table 1. Genes used in this study with PCR primers.

Locus	PCR primers	PCR: thermal cycles: (Annealing temp. in bold)	References
ITS	ITS1/ITS4	(95 °C: 30 s, **51 °C**: 30 s, 72 °C: 1 min) × 35 cycles	White et al. 1990
LSU	LROR/LR5	(95 °C: 45 s, **55 °C**: 30 s, 72 °C: 1 min) × 35 cycles	Vilgalys and Hester 1990
tef1-α	EF1-728F/EF1-986R	(95 °C: 15 s, **55 °C**: 30 s, 72 °C: 1 min) × 35 cycles	Carbone and Kohn 1999
tub2	Bt2a/Bt2b	(95 °C: 30 s, **55 °C**: 30 s, 72 °C: 1 min) × 35 cycles	Glass and Donaldson 1995

Phylogenetic analyses

The sequences obtained were assembled using SeqMan v. 7.1.0 software, and reference sequences from related publications (Phillips et al. 2019; Li et al. 2023; Lin et al. 2023a; Wu et al. 2023) were retrieved from the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov). All sequences generated in this study were submitted to GenBank (Table 2). Sequences were aligned in MAFFT v. 7 at the web server (https://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013; Katoh et al. 2019) and further adjustments and editing of the sequences were made with MEGA v. 6 (Tamura et al. 2013). Maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) were selected to construct phylogenetic trees using PAUP v. 4.0b10, PhyML 3.0, and MrBayes V3.1.2 (Huelsenbeck and Ronquist 2001; Swofford 2003; Silvestro and Michalak 2012). Phylograms were visualized with FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and additional edited with Adobe Illustrator CS v. 5 (Adobe Systems Inc., USA). Maximum-parsimony bootstrap values (MPBP) and maximum-likelihood bootstrap values (MLBP) ≥ 50% and Bayesian posterior probabilities (BYPP) ≥ 0.90 are shown for each tree.

Maximum parsimony analysis was performed using the tree bisection and reconnection (TBR) branch swapping algorithm with a heuristic search option of 1000 random-addition sequences (Swofford 2003). Max trees were set to 5000 branches of zero length, and all parsimonious trees were saved. Other measures calculated were tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency (RC) (Swofford 2003). Maximum likelihood analysis was performed with the GTR GAMMA model of site substitution, including estimation of gamma-distributed rate heterogeneity and a proportion of invariant sites (Guindon et al. 2010). The branch support from MP and ML analysis was evaluated with a bootstrapping (BS) method of 1 000 replicates (Hillis and Bull 1993). The Bayesian inference analysis employing a Markov chain Monte Carlo (MCMC) algorithm was performed with Bayesian posterior probabilities (Rannala and Yang 1996). The model of nucleotide substitution was estimated by MrModeltest v.2.3 (Posada and Crandall 1998), and a weighted Bayesian analysis was considered. Two MCMC chains were run starting from random trees for 1,000,000 generations and stopped when the average standard deviation of split frequencies fell below 0.01; the trees were sampled every 100th generation. The first 25% of trees were discarded as the burn-in phase of each analysis, and the Bayesian posterior probabilities (BPP) were calculated using the remaining 7,500 trees.

Table 2. Isolates of *Aplosporella*, *Dothiorella*, and *Phaeobotryon* used in the molecular analyses in this study. Notes: NA: not applicable, Strains in this study are marked in bold, T: ex-type strains.

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	*tef1-α*	*tub2*	LSU
Aplosporella africana	CBS 121777^{T}	*Acacia mellifera*	Namibia	KF766196	EU101360	NA	NA
A. africana	CBS 1217778^{T}	*Acacia mellifera*	Namibia	EU101316	EU101361	NA	NA
A. artocarpi	CPC 22791^{T}	*Artocarpus heterophyllus*	Thailand	KM006450	KM006481	NA	NA
A. ginkgonis	CFCC 52442^{T}	*Rhus typhina*	China	MH133916	MH133950	NA	NA
A. ginkgonis	CFCC 89661^{T}	*Rhus typhina*	China	KM030583	KM030597	NA	NA
A. ginkgonis	CFCC 70746	*Cotinus coggygria* var. *cinereus*	China	PP188498	PP541796	NA	NA
A. hesperidica	CBS 732.79^{T}	*Citrus aurantium*	Buenos Aires	KX464083	NA	NA	NA
A. hesperidica	CBS 208.37	*Citrus sinensis*	Zimbabwe	JX681069	NA	NA	NA
A. javeedii	CFCC 50054^{T}	*Juniperus chinensis*	China	KP208840	KP208846	NA	NA
A. javeedii	CFCC 50052	*Gleditsia sinensis*	China	KP208838	KP208844	NA	NA
A. javeedii	CFCC 58330	*Populus canadensis*	China	OQ651161	OQ692921	NA	NA
A. javeedii	CFCC 58329	*Populus beijingensis*	China	OQ651162	OQ692922	NA	NA
A. javeedii	CFCC 58412	*Populus alba* var. *pyramidalis*	China	OQ651163	OQ692923	NA	NA
A. javeedii	CFCC 70733	*Styphnolobium japonicum*	China	PP188499	PP541797	NA	NA
A. javeedii	CFCC 70734	*Forsythia suspensa*	China	PP188500	PP541798	NA	NA
A. javeedii	CFCC 70735	*Forsythia suspensa*	China	PP188501	PP541799	NA	NA
A. javeedii	CFCC 70736	*Ulmus pumila*	China	PP188502	PP541800	NA	NA
A. javeedii	CFCC 70737	*Acer truncatum*	China	PP188503	PP541801	NA	NA
A. javeedii	CFCC 70739	*Sambucus williamsii*	China	PP188504	PP541802	NA	NA
A. javeedii	CFCC 70740	*Acer miyabei*	China	PP188505	PP541803	NA	NA
A. javeedii	CFCC 70741	*Lagerstroemia indica*	China	PP188506	PP541804	NA	NA
A. javeedii	CFCC 70742	*Xanthoceras sorbifolium*	China	PP188507	PP541805	NA	NA
A. javeedii	CFCC 70744	*Syringa vulgaris*	China	PP188508	PP541806	NA	NA
A. javeedii	CFCC 70745	*Ulmus pumila*	China	PP188509	PP541807	NA	NA
A. macropycnidia	CGMCC 3.17725^{T}	*Cerasus yedoensis*	China	KT343648	KX011176	NA	NA
A. macropycnidia	CGMCC 3.17726	*Cerasus yedoensis*	China	KT343649	KX011177	NA	NA
A. papillata	CBS 121780^{T}	*Acacia tortillas*	South Africa	EU101328	EU101373	NA	NA
A. papillata	CBS 121781	*Acacia tortillas*	South Africa	EU101329	EU101374	NA	NA
A. prunicola	CBS 121167^{T}	*Prunus persica* var. *nucipersica*	South Africa	KF766147	NA	NA	NA
A. prunicola	STE-U 6326	*Prunus persica* var. *nucipersica*	South Africa	EF564375	NA	NA	NA
A. sophorae	CPC 29688^{T}	*Sophora microphylla*	New Zealand North	KY173388	NA	NA	NA
A. thailandica	MFLU 16-0615^{T}	Dead stems	Thailand	KX423536	KX423537	NA	NA
A. yalgorensis	MUCC511^{T}	*Acacia cochlearis*	Australia	EF591926	EF591977	NA	NA
A. yalgorensis	MUCC512	*Eucalyptus gomphocephala*	Australia	EF591927	EF591978	NA	NA
A. yanqingensis	CFCC 58791^{T}	*Platycladus orientalis*	China	OQ651164	OQ692924	NA	NA
A. yanqingensis	CFCC 58792^{T}	*Platycladus orientalis*	China	OQ651165	OQ692925	NA	NA
A. yanqingensis	CFCC 70743	*Acer truncatum*	China	PP188510	PP541808	NA	NA
A. yanqingensis	CFCC 70738	*Acer truncatum*	China	PP188511	PP541809	NA	NA
Alanomyces indica	CBS 134264^{T}	Soil	India	HF563622	AB872219	NA	NA
Dothiorella alpina	CGMCC 3-18001^{T}	*Platycladus orientalis*	China	KX499645	KX499651	NA	NA
Do. acacicola	CBS 141295^{T}	*Acacia mearnsii*	Réunion	KX228269	KX228376	NA	NA
Do. acericola	KUMCC 18-0137^{T}	*Acer palmatum*	China	MK359449	MK361182	NA	NA
Do. acericola	CFCC 70755	*Forsythia suspensa*	China	PP188520	PP766251	PP566659	NA
Do. acericola	CFCC 70760	*Ginkgo biloba*	China	PP188521	PP766252	PP566660	NA
Do. acericola	CFCC 70761	*Syringa oblata*	China	PP188522	PP766253	PP566661	NA

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	*tef1-α*	*tub2*	LSU
Do. albiziae	MFLUCC 22-0057^{T}	*Albizia lebbeck*	Thailand	ON751762	ON799588	ON799590	NA
Do. alpina	CFCC 58299^{T}	*Populus szechuanica*	China	OQ651166	OQ692932	OQ692926	NA
Do. americana	CBS 128309^{T}	*Vitis species and Vitis vinifera*	USA: Missouri	HQ288218	HQ288262	HQ288297	NA
Do. baihuashanensis	CFCC 58549^{T}	*Juniperus chinensis*	China	OQ651167	OQ692933	OQ692927	NA
Do. baihuashanensis	CFCC 58788^{T}	*Juniperus chinensis*	China	OQ651168	OQ692934	OQ692928	NA
Do. brevicollis	CBS 130411 = CMW 36463^{T}	*Acacia karroo*	South Africa	JQ239403	JQ239390	JQ239371	NA
Do. californica	CBS 119635	*Laurus nobilis*	Turkey	MT587396	MT592108	MT592579	NA
Do. californica	CBS 141587^{T}	*Umbellularia californica*	USA	KX357188	KX357211	KX357165	NA
Do. camelliae	CMGCC 3.24158^{T}	*Camellia oleifera*	China	OQ190531	OQ241464	OQ275064	NA
Do. capri-amissi	CBS 121763 = CMW 25403 = CAMS 1158^{T}	*Acacia erioloba*	South Africa	EU101323	EU101368	KX464850	NA
Do. capri-amissi	CBS 121878 = CMW 25404 = CAMS 1159^{T}	*Acacia erioloba*	South Africa	EU101324	EU101369	KX464851	NA
Do. casuarinae	CBS 120688 = CMW 4855^{T}	*Casuarina* sp.	Australia	DQ846773	DQ875331	DQ875340	NA
Do. casuarinae	CBS 120689 = CMW 4856	*Casuarina* sp.	Australia	DQ846772	DQ875332	DQ875339	NA
Do. casuarinae	CBS 120690 = CMW 4857	*Casuarina* sp.	Australia	DQ846774	DQ875333	DQ875341	NA
Do. citricola	CBS 124728 = ICMP 16827	*Citrus sinensis*	New Zealand	EU673322	EU673289	KX464852	NA
Do. citricola	CBS 124729 = ICMP 16828^{T}	*Citrus sinensis*	New Zealand	EU673323	EU673290	KX464853	NA
Do. citrimurotticola	BE5 = CGMCC3.20392^{T}	*Citrus unshiu*	China	MW880663	MW884166	MW884195	NA
Do. citrimurotticola	BE8 = CGMCC3.20394	*Citrus reticulatachen* × *C. sinensis*	China	MW880661	MW884164	MW884193	NA
Do. diospyricola	CBS 145972	*Diospyros mespiliformis*	South Africa	MT587398	MT592110	MT592581	NA
Do. dulcispinae	CBS 121764 = CMW 25406 = CAMS 1159	*Acacia mellifera*	Namibia	EU101299	EU101344	KX464854	NA
Do. dulcispinae	CBS 130413 = CMW 36460^{T}	*Acacia karroo*	South Africa	JQ239400	JQ239387	JQ239373	NA
Do. eriobotryae	CBS 140852^{T}	*Eriobotrya japonica*	Spain	KT240287	KT240262	MT592582	NA
Do. franceschinii	CBS 147722	*Rhamnus alaternus*	Italy	OP999677	OQ067247	NA	NA
Do. guttulata	MFLUCC 17-0242	*Alnus glutinosa*	Italy	KY797637	NA	NA	NA
Do. heterophyllae	CMW 46458^{T}	*Acacia heterophylla*	Réunion	MN103794	MH548348	MH548324	NA
Do. hortiarborum	CFCC 70756^{T}	*Fraxinus chinensis*	China	PP188523	PP723042	PP566662	NA
Do. hortiarborum	CFCC 70757	*Fraxinus chinensis*	China	PP188524	PP723043	PP566663	NA
Do. hortiarborum	CFCC 70758	*Lagerstroemia indica*	China	PP188525	PP723044	PP566664	NA
Do. hortiarborum	CFCC 70759	*Lagerstroemia indica*	China	PP188526	PP723045	PP566665	NA
Do. iberica	CBS 113188 = DA-1	*Quercus suber*	Spain	AY573198	EU673278	EU673097	NA
Do. iberica	CBS 113189 = DE-14	*Quercus ilex*	Spain	AY573199	AY573230	KX464855	NA
Do. iberica	CBS 115041 = CAP 145^{T}	*Quercus ilex*	Spain	AY573202	AY573222	EU673096	NA
Do. irannica	CBS 124722 = CJA 153 = IRAN 1587C^{T}	*Olea europaea*	Iran, Golestan	KC898231	KC898214	KX464856	NA
Do. koae	CMW 48017^{T}	*Acacia koa*	Hawaiian Is.	MH447652	MH548338	MH548327	NA
Do. lampangensis	MFLUCC 18-0232^{T}	Rutaceae	Thailand	MK347758	MK340869	MK412874	NA
Do. longicollis	CBS 122066 = CMW 26164	*Terminalia* sp.	Australia	EU144052	EU144067	KX464857	NA
Do. longicollis	CBS 122067 = CMW 26165	*Lysiphyllum cunninghamii*	Australia	EU144053	EU144068	KX464858	NA
Do. longicollis	CBS 122068 = CMW 26166^{T}	*Lysiphyllum cunninghamii*	Australia	EU144054	EU144069	KF766130	NA
Do. magnoliae	CFCC51563^{T}	*Magnolia grandiflora*	China	KY111247	KY213686	NA	NA
Do. mangifericola	CBS 124727^{T}	*Mangifera indica*	Iran	KC898221	KX464614	NA	NA
Do. mangifericola	IRAN 1584C	*Mangifera indica*	Iran	MT587407	MT592119	NA	NA
Do. moneti	WAC 13154 = MUCC 505^{T}	*Acacia rostellifera*	Australia	EF591920	EF591971	EF591954	NA
Do. neclivorem	DAR 80992^{T}	*Vitis vinifera*	Australia	KJ573643	KJ573640	KJ577551	NA
Do. oblonga	CBS 121765 = CMW 25407 = CAMS 1162^{T}	*Acacia mellifera*	South Africa	EU101300	EU101345	KX464862	NA
Do. oblonga	CBS 121766 = CMW 25408 = CAMS 1163	*Acacia mellifera*	South Africa	EU101301	EU101346	KX464863	NA
Do. obovata	MFLUCC22-0058^{T}	*Pavonia odorata*	Thailand	ON751763	ON799589	ON799591	NA

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	*tef1-α*	*tub2*	LSU
Do. omnivora	CBS 124717 = CJA 214 = IRAN 1570C	*Juglans regia*	Iran	KC898233	KC898216	KX464865	NA
Do. omnivora	CBS 392.80	–	France	KX464133	KX464626	KX464897	NA
Do. omnivora	CBS 124716 = CJA 241 = IRAN 1573C	*Juglans regia*	Iran	KC898232	KC898215	KX464864	NA
Do. omnivora	CBS 242.51	–	Italy	EU673317	EU673284	EU673105	NA
Do. omnivora	CBS 188.87	*Juglans regia*	France	EU673316	EU673283	EU673119	NA
Do. parva	CBS 124720 = CJA 27 = IRAN 1579C^{T}	*Corylus* sp.	Iran	KC898234	KC898217	KX464866	NA
Do. parva	CBS 124721 = CJA 35	*Corylus* sp.	Iran	KX464123	KX464615	KX464867	NA
Do. parva	CBS 125580	*Corylus avellana*	Austria	KX464124	KX464616	KX464868	NA
Do. plurivora	CBS 124724 = CJA 254 = IRAN 1557C^{T}	*Citrus* sp.	Iran	KC898225	KC898208	KX464874	NA
Do. pretoriensis	CBS 130404 = CMW 36480^{T}	*Acacia karroo*	South Africa	JQ239405	JQ239392	JQ239376	NA
Do. prunicola	CBS 124723 = CAP 187 = IRAN 1541C^{T}	*Prunus dulcis*	Portugal	EU673313	EU673280	EU673100	NA
Do. rhamni	MFLUCC 14-0902^{T}	*Rhamnus cathartica*	South European Russia	MF398893	MF398945	NA	NA
Do. rosulata	CBS 121760 = CMW 25389 = CAMS 1444^{T}	*Acacia karroo*	Namibia	KF766227	EU101335	KX464877	NA
Do. rosulata	CBS 121761 = CMW 25392 = CAMS 1147	*Acacia mellifera*	South Africa	EU101293	EU101338	KX464878	NA
Do. rosulata	CBS 121762 = CMW 25395 = CAMS 1150	*Acacia mellifera*	South Africa	EU101319	EU101364	KX464879	NA
Do. rosulata	CBS 500.72	*Medicago sativa*	South Africa	EU673318	EU673285	EU673118	NA
Do. santali	WAC 13155 = MUCC 509^{T}	*Santalum acuminatum*	Australia	EF591924	EF591975	EF591958	NA
Do. saprophytica	MFLUCC 23-0210	–	Thailand	OR527239	OR532455	OR532454	NA
Do. sarmentorum	IMI 63581b	*Ulmus* sp.	UK: England	AY573212	AY573235	EU673102	NA
Do. sempervirentis	IRAN 1581C = CBS 124719	*Cupressus sempervirens*	Iran	KC898237	KC898220	KX464885	NA
Do. sempervirentis	IRAN 1583C = CBS 124718 = CJA 264^{T}	*Cupressus sempervirens*	Iran	KC898236	KC898219	KX464884	NA
Do. sp.	CBS 121783 = CMW 25432 = CAMS 1187	*Acacia mearnsii*	South Africa	EU101333	EU101378	KX464859	NA
Do. sp.	CBS 121784 = CMW 25430 = CAMS 1185	*Acacia mearnsii*	South Africa	EU101331	EU101376	KX464860	NA
Do. sp.	CBS 121785 = CMW 25433 = CAMS 1188	*Acacia mearnsii*	South Africa	EU101334	EU101379	KX464861	NA
Do. striata	CBS 124730 = ICMP 16819	*Citrus sinensis*	New Zealand	EU673320	EU673287	EU673142	NA
Do. striata	CBS 124731 = ICMP 16824^{T}	*Citrus sinensis*	New Zealand	EU673321	EU673288	EU673143	NA
Do. styphnolobii	Cr01^{T}	*Styphnolobium japonicum*	Crym	MH880849	MK069594	NA	NA
Do. symphoricarpicola	CPC 33923^{T}	*Symphoricarpos*	Italy	MT587414	MT592126	MT592606	NA
Do. tectonae	MFLUCC18-0232^{T}	*Tectona grandis*	Thailand	KM396899	KM409637	KM510357	NA
Do. thailandica	CBS 133991 = CPC 21557 = MFLUCC 11-0438	*Dead bamboo culm*	Thailand	JX646796	JX646861	JX646844	NA
Do. thripsita	CBS 125445 = BRIP 51876a^{T}	*Acacia harpophylla*	Australia	KJ573642	KJ573639	KJ577550	NA
Do. ulmacea	CBS 141414^{T}	*Ulmus laevis*	Germany	MT587415	MT592127	MT592608	NA
Do. uruguayensis	CBS 124908 = CMW 26763^{T}	*Hexachlamis edulis*	Uruguay	EU080923	EU863180	KX464886	NA
Do. vidmadera	CBS 621.74	*Pyrus communis*	Switzerland	KX464129	KX464621	KX464887	NA
Do. vidmadera	CBS 725.79^{T}	*Pyrus malus*	Switzerland	KX464130	KX464622	KX464888	NA
Do. vinea-gemmae	DAR 81012^{T}	*Vitis vinifera*	Australia	KJ573644	KJ573641	KJ577552	NA
Do. viticola	CBS 117009^{T}	*Vitis vinifera*	Spain	AY905554	AY905559	EU673104	NA
Do. westralis	WA10NO01^{T}	*Vitis vinifera*	Australia	HM009376	HM800511	NA	NA
Do. yunnana	CGMCC 3-17999^{T}	*Camellia* sp.	China	KX499643	KX499649	NA	NA
Do. yunnana	CGMCC 3-18000	*Camellia* sp.	China	KX499644	KX499650	NA	NA
Do. zanthoxyli	CMGCC 3.24159^{T}	*Zanthoxylum bungeanum*	Sichuan	OQ190536	OQ241468	OQ275069	NA

Species	Strain	Host	Origin	GenBank accession numbers			
				ITS	*tef1-α*	*tub2*	LSU
Neofusicoccum luteum	CBS 562.92^{T}	*Actinidia deliciosa*	New Zealand	MH862376	KX464690	KX464968	NA
Neofusicoccum parvum	CMW 9081^{T}	*Populus nigra*	New Zealand	AY236943	AY236888	AY236917	NA
Phaeobotryon aplosporum	CFCC 53774	*Syzygium aromaticum*	China	MN215836	MN205996	NA	MN215871
P. aplosporum	CFCC 53775^{T}	*Rhus typhina*	China	MN215837	NA	NA	MN215872
P. aplosporum	CFCC 53776	*Rhus typhina*	China	MN215838	MN205997	NA	MN215873
P. aplosporum	CFCC 58596	*Juglans mandshurica*	China	OQ651169	NA	NA	OQ652540
P. aplosporum	CFCC 58784	*Juglans mandshurica*	China	OQ651170	NA	NA	OQ652541
P. cupressi	CBS 124700 = IRAN 1455C^{T}	*Cupressus sempervirens*	Iran	FJ919672	FJ919661	NA	KX464538
P. cupressi	CBS 124701 = IRAN 1458C	*Cupressus sempervirens*	Iran	FJ919671	FJ919660	NA	KX464539
P. fraxini	CFCC 70762^{T}	*Fraxinus chinensis*	China	PP188527	PP505782	NA	PP177348
P. fraxini	CFCC 70763	*Fraxinus chinensis*	China	PP188528	PP505783	NA	PP177349
P. juniperi	JU001^{T}	*Juniperus formosana*	China	OP941637	OP948218	NA	OP941644
P. juniperi	JU005	*Juniperus formosana*	China	OP941638	OP948219	NA	OP941645
P. mali	XJAU 2930^{T}	*Malus pumila*	China	MW326854	MW509519	NA	MW367101
P. mali	XJAU 2772	*Juglans regia*	China	MW326853	MW509520	NA	MW367094
P. mali	XJAU 2782	*Malus 'Royalty'*	China	MW326852	MW509516	NA	MW367092
P. mali	XJAU 3094	*Elaeagnus angustifolia*	China	MW326858	MW509517	NA	MW367100
P. mali	XJAU 3100	*Rhus typhina*	China	MW326878	MW509518	NA	MW367093
P. mamane	CBS 122980 = CPC 12440^{T}	*Sophora chrysophylla*	USA	EU673332	EU673298	NA	EU673248
P. mamane	CPC 12442	*Sophora chrysophylla*	USA	EU673333	EU673299	NA	DQ377899
P. negundinis	CAA 797	*Acer negundo*	Russia	KX061513	KX061507	NA	NA
P. negundinis	CAA 798	*Ligustrum vulgare*	Russia	KX061514	KX061508	NA	NA
P. negundinis	CAA 799	*Forsythia intermedia*	Russia	KX061515	KX061509	NA	NA
P. negundinis	CPC 33384	*Acer nugundo*	Ukraine	MT587542	MT592276	NA	MT587323
P. negundinis	CPC 33388	*Dead stem*	Ukraine	MT587543	MT592277	NA	MT587324
P. negundinis	CPC 34752	*Acer negundo*	Ukraine	MT587544	MT592278	NA	MT587325
P. negundinis	MFLUCC 15-0436^{T}	*Acer negundo*	Russia	KU820970	KU853997	NA	NA
P. platycladi	CFCC 58799^{T}	*Platycladus orientalis*	China	OQ651172	OQ692930	NA	OQ652543
P. platycladi	CFCC 58800	*Platycladus orientalis*	China	OQ651173	OQ692931	NA	OQ652544
P. rhoinum	CFCC 52449	*Rhus typhina*	China	MH133923	MH133957	NA	MH133940
P. rhoinum	CFCC 52450^{T}	*Rhus typhina*	China	MH133924	MH133958	NA	MH133941
P. rhois	CFCC 89662 = CCTCC AF2014017^{T}	*Rhus typhina*	China	KM030584	KM030598	NA	KM030591
P. rhois	CFCC 89663 = CCTCC AF2014016	*Rhus typhina*	China	KM030585	KM030599	NA	KM030592
P. rhois	CFCC 58679^{T}	*Populus alba* var. *pyramidalis*	China	OQ651171	OQ692929	NA	OQ652542
P. spiraeae	CFCC 53925^{T}	*Spiraea salicifolia*	China	OM049420	NA	NA	OM0049432
P. spiraeae	CFCC 53926	*Spiraea salicifolia*	China	OM049421	NA	NA	OM0049433
P. spiraeae	CFCC 53927	*Spiraea salicifolia*	China	OM049422	NA	NA	OM0049434
P. ulmi	94-13	*Ulmus pumila*	USA	AF243398	NA	NA	NA
P. ulmi	CBS 114123 = UPSC 2552	*Ulmus glabra*	Sweden	MT587539	MT592273	NA	MT587320
P. ulmi	CBS 138854 = CPC 24264^{T}	*Ulmus laevis*	Germany	MT587540	MT592274	NA	MT587321
P. ulmi	CBS 123.30 = ATCC 24443	*Ulmus* sp.	USA	KX464232	KX464766	NA	DQ377861
P. ulmi	CBS 174.63	*Ulmus glabra*	Finland	MT587541	MT592275	NA	MT587322
P. ulmi	CMH 299	*House dust*	USA	KF800390	NA	NA	NA
P. ulmi	PB_11f	*Ulmus glabra*	Poland	MK134682	NA	NA	NA
Alanphillipsia aloeicola	CBS 138896 = CPC 23674^{T}	*Aloe* sp.	South Africa	KP004444	MT592027	NA	KP004472

Result

Phylogenetic analysis

The BLAST results indicated that the 23 isolates resided in *Aplosporella*, *Dothiorella*, and *Phaeobotryon* (14 for *Aplosporella*, 7 for *Dothiorella*, and 2 for *Phaeobotryon*). Separate phylogenetic trees for each of the three genera were constructed in this study.

In *Aplosporella*, the combined ITS and *tef1-α* dataset consists of 944 characters, including alignment gaps (508 for ITS and 436 for *tef1-α*), of which 794 are constant and 60 are variable parsimony uninformative characters. MP analysis with the remaining 90 parsimony-informative characters resulted in one equally parsimonious tree: tree length (TL) = 230; consistency index (CI) = 0.817; retention index (RI) = 0.896; and rescaled consistency index (RC) = 0.732. In ML analysis based on the combined gene dataset, the matrix had 193 distinct alignment patterns. Estimated base frequencies are as follows: A = 0.217607, C = 0.264598, G = 0.259539, T = 0.258256, AC = 2.784746, AG = 2.845183, AT = 1.353935, CG = 1.848853, CT = 4.935430, GT = 1.000000, gamma distribution shape parameter: α = 0.157110, and likelihood value of ln: -2 499.855852. The maximum likelihood (ML) and Bayesian methods (BI) for phylogenetic analyses have the same topology and terminal clades. Fourteen isolates were distributed in *Aplosporella*, aggregated with three known species, *A. javeedii*, *A. yanqingensis*, and *A. ginkgonis*, separately (Fig. 2). The single gene tree for ITS and *tef1-α* of *Aplosporella* is shown in Suppl. material 1.

In *Dothiorella*, sequences of the combined ITS, *tef1-α*, and *tub2* were aligned; the dataset consists of 1,319 characters, including alignment gaps (534 for ITS, 369 for *tef1-α*, and 416 for *tub2*), of which 905 are constant and 107 are variable parsimony uninformative characters. MP analysis with the remaining 307 parsimony-informative characters resulted in one equally parsimonious tree: tree length (TL) = 1,282; consistency index (CI) = 0.477; retention index (RI) = 0.824; and rescaled consistency index (RC) = 0.394. In ML analysis based on the combined gene dataset, the matrix had 601 distinct alignment patterns. Estimated base frequencies are as follows: A = 0.206208, C = 0.312741, G = 0.250328, T = 0.230723, AC = 0.833804, AG = 2.174710, AT = 1.041501, CG = 0.791470, CT = 3.735830, GT = 1.000000, gamma distribution shape parameter: α = 0.215045, and likelihood value of ln: -8 567.497788. Three of the seven isolates were of the known species *Dothiorella acericola*, and the other four isolates formed a separate clade for designation as new species based on phylogenetic analysis (Fig. 3). The single gene tree for ITS, *tef1-α*, and *tub2* of *Dothiorella* is shown in Suppl. material 2.

In *Phaeobotryon*, the combined ITS, LSU, and *tef1-α* dataset consists of 1,394 characters, including alignment gaps (494 for ITS, 333 for LSU, and 567 for *tef1-α*), of which 1,218 are constant and 56 are variable parsimony uninformative characters. MP analysis with the remaining 120 parsimony-informative characters resulted in one equally parsimonious tree: tree length (TL) = 259; consistency index (CI) = 0.799; retention index (RI) = 0.913; and rescaled consistency index (RC) = 0.730. In ML analysis based on the combined gene dataset, the matrix had 239 distinct alignment patterns. Estimated base frequencies are as follows: A = 0.224820, C = 0.266099, G = 0.277247, T = 0.231833, AC = 0.602998, AG = 2.181745, AT = 0.500445, CG = 0.607508, CT = 4.549533, GT = 1.000000, gamma distribution shape parameter: α = 0.020014, and likelihood

value of ln: -3 357.887099. Eight isolates were assigned to *Phaeobotryon*, one isolate aggregated with *P. mali*, and two isolates stood alone, not branching off from known species, representing a new species (Fig. 4). The single gene tree for ITS, LSU, and *tef1-α* of *Phaeobotryon* is shown in Suppl. material 3.

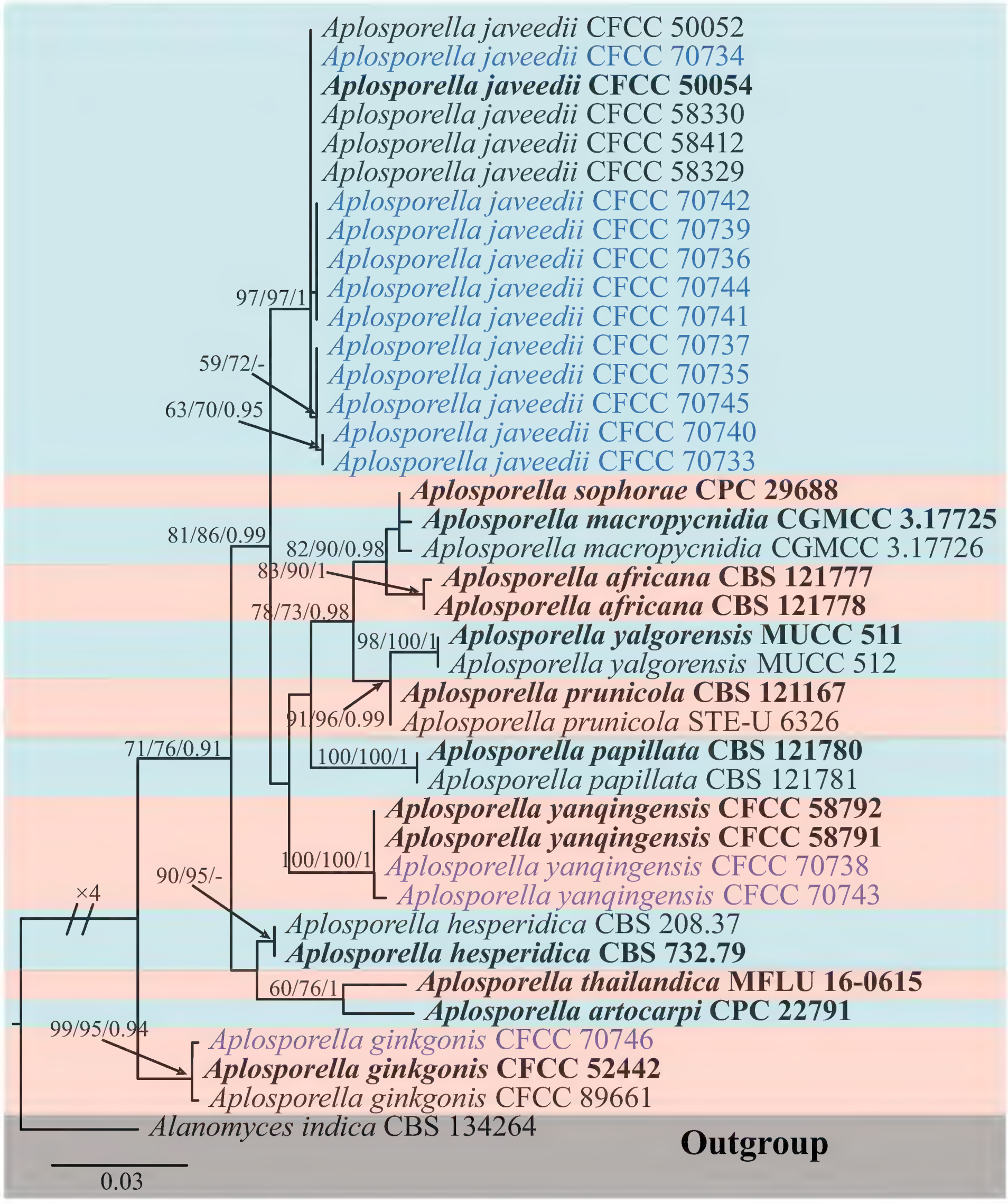

Figure 2. Phylogram generated from RAxML analysis based on ITS with *tef1-α* sequence data of *Aplosporella* isolates. The tree was rooted in *Alanomyces indica* (CBS 134264). The MP, ML (≥ 50%), and BI (≥ 0.9) bootstrap supports are given near the nodes, respectively. Isolates from this study are marked in blue, and ex-type strains are marked in bold.

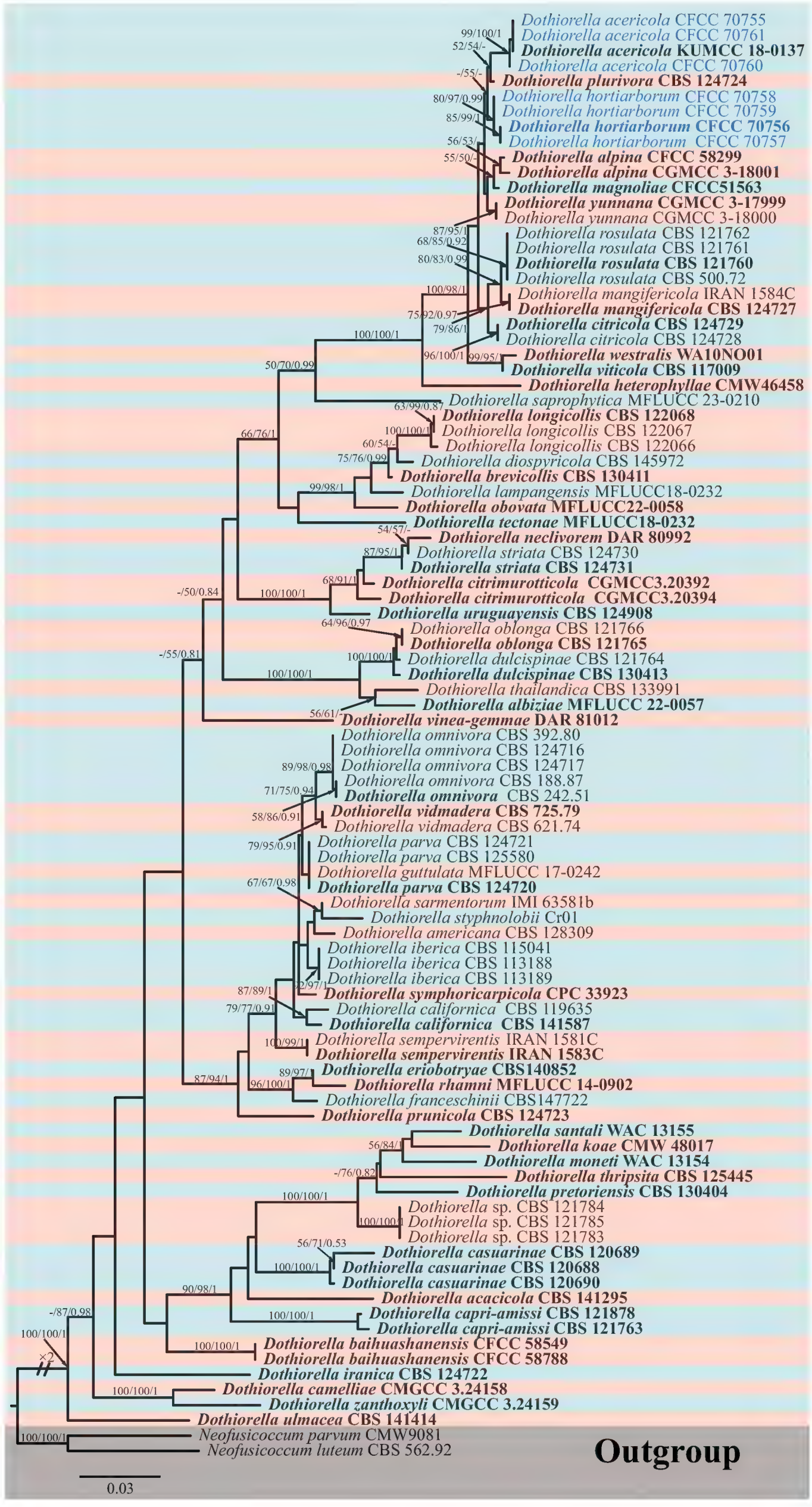

Figure 3. Phylogram generated from RAxML analysis based on ITS, *tef1-a*, and *tub2* sequence data of *Dothiorella* isolates. The tree was rooted in *Neofusicoccum luteum* (CBS 562.92) and *Neofusicoccum parvum* (CMW9081). The MP, ML (≥ 50%), and BI (≥ 0.9) bootstrap supports are given near the nodes, respectively. Isolates from this study are marked in blue, and ex-type strains are marked in bold.

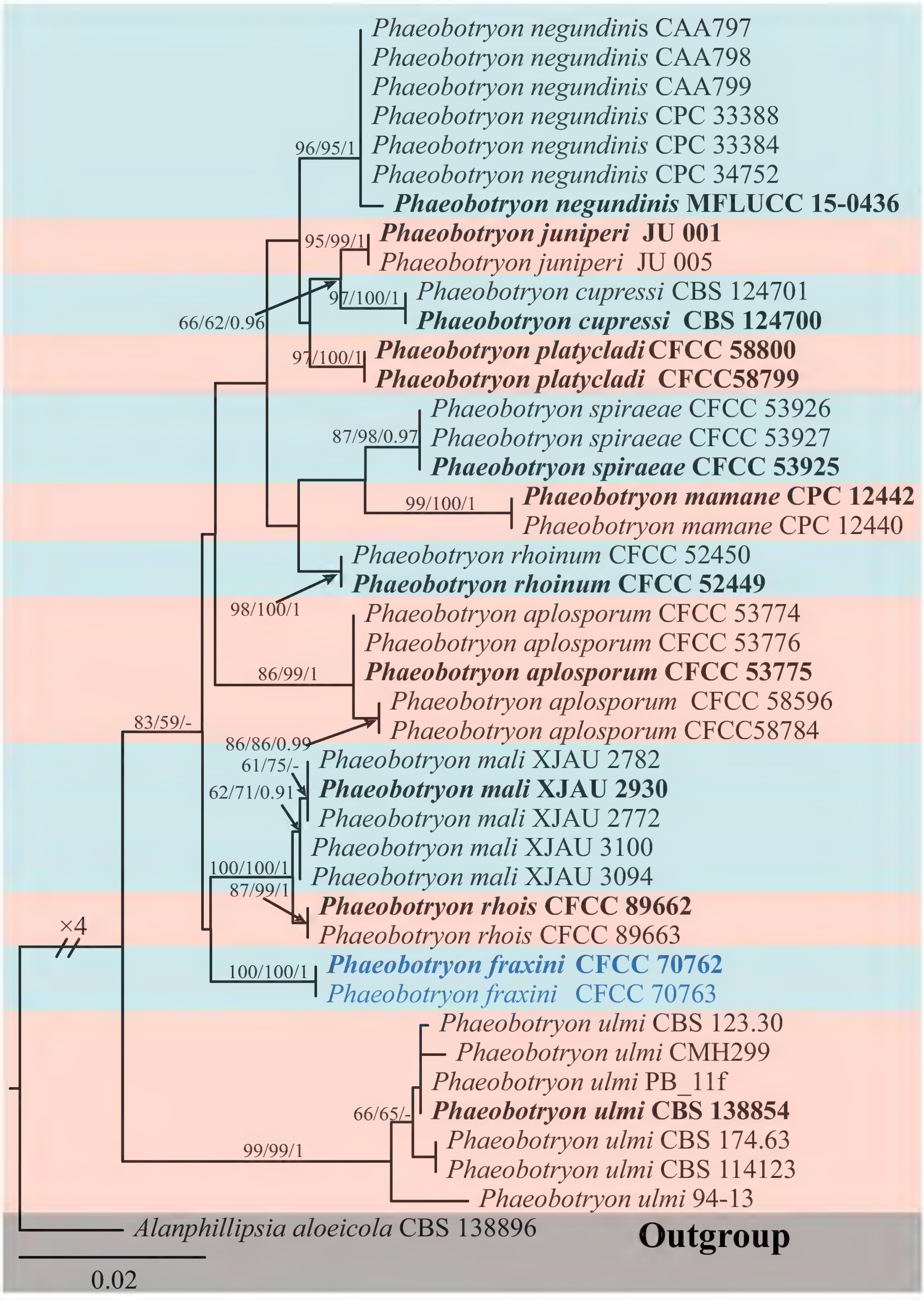

Figure 4. Phylogram generated from RAxML analysis based on ITS, LSU, and *tef1-α* sequence data of *Phaeobotryon* isolates. The tree was rooted in *Alanphillipsia aloeicola* (CBS 138896). The MP, ML (≥ 50%), and BI (≥ 0.9) bootstrap supports are given near the nodes, respectively. Isolates from this study are marked in blue, and ex-type strains are marked in bold.

Taxonomy

***Aplosporella ginkgonis* C.M. Tian, Z. Du & K.D. Hyde, Mycosphere 8(2): 1249 (2017)**

Description. See Du et al. 2017.

Material examined. CHINA, Beijing City, Tongzhou District, Majuqiao Wetland Park, 39°46'12"N, 116°37'12"E, on the disease branches of *Cotinus coggygria* var. *cinereus*, 2 May 2023, Y.Y. Wu, BJFC-S1931, living culture CFCC 70746.

Notes. *Aplosporella ginkgonis* was first reported in Gansu Province, China, causing canker and dieback disease in *Ginkgo biloba* and *Morus alba* (Du et al. 2017). Zhu et al. (2018) and Li et al. (2023) discovered the species on *Rhus typhina* and *Zanthoxylum bungeanum*, respectively, extending its host range. In the present study, one isolate (CFCC 70746) was identified as *A. ginkgonis* based on the phylogenetically highly supported clade with 99% MP, 95% ML, and 0.94 BYPP values (Fig. 2) and morphological characteristics. This is the first report of *A. ginkgonis* on *Cotinus coggygria* var. *cinereus*.

***Aplosporella javeedii* Jami, Gryzenh., Slippers & M.J. Wingf., Fungal Biology 118(2): 174 (2013)**

Description. See Fan et al. 2015.

Material examined. CHINA, Beijing City, Tongzhou District, Hougezhuang Plain Forest, 29°50'24"N, 116°54'00"E, on the dead branches of *Styphnolobium japonicum*, 8 April 2023, C.M. Tian, S.J. Li & Y.Y. Wu, BJFC-S1932, living culture CFCC 70733; *ibid*. on the dead branches of *Forsythia suspensa*, BJFC-S1933, living culture CFCC 70734; *ibid*. on the dead branches of *Forsythia suspensa*, BJFC-S1934, living culture CFCC 70735; *ibid*. on the dead branches of *Ulmus pumila*, BJFC-S1935, living culture CFCC 70736; CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, from branches of *Acer truncatum*, 12 April 2023, C.M. Tian, Y.M. Liang, C. Peng, Y. Hu & Y.Y. Wu, BJFC-S1936, living culture CFCC 70737; CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, on the dead branches of *Sambucus williamsii*, 19 April 2023, C.M. Tian, C. Peng, R. Yuan, M.W. Zhang & Y.Y. Wu, BJFC-S1937, living culture CFCC 70739; *ibid*. on the dead branches of *Acer miyabei*, BJFC-S1938, living culture CFCC 70740; *ibid*. on the dead branches of *Lagerstroemia indica*, BJFC-S1939, living culture CFCC 70741; *ibid*. on the dead branches of *Xanthoceras sorbifolium*, BJFC-S1940, living culture CFCC 70742; China, Beijing City, Tongzhou District, Majuqiao Wetland Park, 39°46'12"N, 116°37'12"E, from branches of *Syringa vulgaris*, 2 May 2023, Y.Y. Wu, BJFC-S1941, living culture CFCC 70744, *ibid*. on the dead branches of *Ulmus pumila*, BJFC-S1942, living culture CFCC 70745.

Notes. *Aplosporella javeedii* was initially reported on *Celtis africana* and *Searsia lancea* in South Africa (Jami et al. 2014). Fan et al. (2015a) recorded this species in China for the first time, associating it with the canker or dieback disease

of five hosts: *Albizia julibrissin*, *Broussonetia papyrifera*, *Gleditsia sinensis*, *Juniperus chinensis*, and *Styphnolobium japonicum*. *Aplosporella javeedii* is widespread on host plants of more than 10 families (Fan et al. 2015a; Zhu et al. 2018; Pan et al. 2019; Lin et al. 2023a). In this study, we report new host records for this species, including *Acer miyabei*, *Acer truncatum*, *Forsythia suspensa*, *Lagerstroemia indica*, *Sambucus williamsii*, *Syringa vulgaris*, *Ulmus pumila*, and *Xanthoceras sorbifolium*.

***Aplosporella yanqingensis* L. Lin & X.L. Fan, MycoKeys 97: 9 (2023)**

Description. See Lin et al. 2023a.

Material examined. CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, on the dead branches of *Acer truncatum*, 12 April 2023, C.M. Tian, Y.M. Liang, C. Peng, Y. Hu & Y.Y. Wu, BJFC-S1943, living culture CFCC 70743; *ibid*. BJFC-S1944, living culture CFCC 70738.

Notes. *Aplosporella yanqingensis* was first discovered on the branches of *Platycladus orientalis* in Beijing (Lin et al. 2023a). In this study, the two isolates (CFCC 70738 and CFCC 70743) from *Acer truncatum* formed a clade with 100% MP, 100% ML, and 1.00 BYPP values in the multi-locus phylogenetic tree with *A. yanqingensis* (Fig. 2). Compared with the description of Lin et al. (2023a), this study has shorter conidia and thinner conidiogenous cells (11.0–16.5 × 6.0–9.0 µm vs. 16.0–21.5 × 6.0–9.5 µm and 5.0–20.5 × 1.0–2.0 µm vs. 6.0–13.5 × 2.0–3.0 µm). Thus, these isolates were identified as *A. yanqingensis*, and herewith we are providing a new host record for *A. yanqingensis*, *Acer truncatum*.

***Dothiorella acericola* Phookamsak, Tennakoon & K.D. Hyde, Fungal Diversity 95: 78 (2019)**

Description. See Pan et al. 2021.

Material examined. CHINA, Beijing City, Tongzhou District, Hougezhuang Plain Forest, 29°50'24"N, 116°54'00"E, on the dead branches of *Forsythia suspensa*, 8 April 2023, C.M. Tian, S.J. Li & Y.Y. Wu, BJFC-S1948, living culture CFCC 70755; CHINA, Beijing City, Tongzhou District, Majuqiao Wetland Park, 39°46'12"N, 116°37'12"E, on the dead branches of *Ginkgo biloba*, 2 May 2023, Y.Y. Wu, BJFC-S1949, living culture CFCC 70760; *ibid*. on the dead branches of *Syringa oblata*, BJFC-S1950, living culture CFCC 70761.

Notes. Based on phylogenetic analyses (Fig. 3), three isolates in this study clustered with *Dothiorella acericola* and formed a clade with 99% MP, 100% ML, and 1.00 BYPP values. *Dothiorella acericola* is reported to be associated with the canker disease of *Acer palmatum* in China (Phookamsak et al. 2019). Pan et al. (2021, 2023) found that *Do. acericola* infests *Ziziphus jujuba* and *Koelreuteria paniculata* branches. The fungus was also recorded on dead branches of *Euonymus japonicus* (Lin et al. 2023b). This is the first discovery of this fungus in the host families Oleaceae and Ginkgoaceae.

Dothiorella hortiarborum **Y.Y. Wu & C.M. Tian, sp. nov.**
MycoBank No: 851826
Fig. 5

Etymology. "Hort" means "garden," and "arbor" means "tree" in Latin. Collected from *Fraxinus chinensis* and *Lagerstroemia indica*, both of which are landscaping and greening trees.

Holotype. CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, on the dead branches of *Fraxinus chinensis*, 19 April 2023, C.M. Tian, C. Peng, R. Yuan, M.W. Zhang & Y.Y. Wu (holotype BJFC-S1951, ex-type cultures CFCC 70756).

Description. ***Sexual morph*****:** Not observed. ***Asexual morph*****: *Conidiomata*** pycnidial, scattered to aggregated, immersed to semi-immersed in bark, globose to subglobose, dark gray to black, unilocular, 260–450 µm diam. ***Disc*** black, ovoid, 310–330 µm diam. ***Ostioles*** single, light gray, circular, central, papillate, 30–45 µm diam. ***Locules*** single, black, oval, 100–380 µm, ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*****:** hyaline, smooth, thin-walled, holoblastic, cylindrical to subcylindrical, 4.5–11.0 × 2.0–4.0 µm (av. ± S.D.= 6.8 ± 1.3 × 2.9 ± 0.5 µm). ***Conidia*** initially hyaline, then producing light yellow pigmentation, uneven surface, thick-walled, dark brown when matrues, 1-septate, constricted at the septum, smooth, ovoid with a broadly rounded apex, truncate base. 10.0–19.0 × 6.0–11.0 µm (av. ± S.D.= 14.9 ± 2.6 × 8.1 ± 1.0 µm).

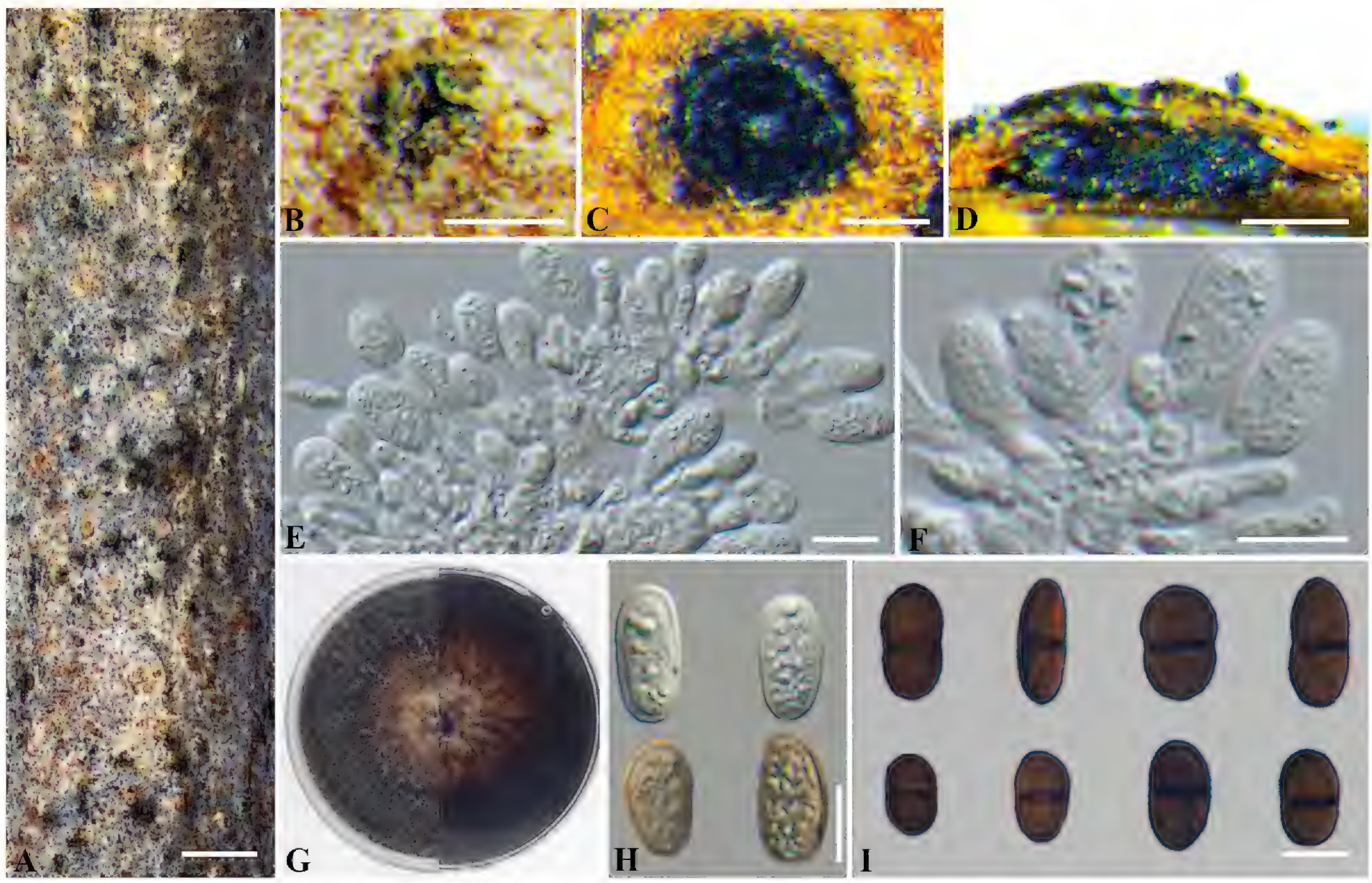

Figure 5. *Dothiorella hortiarborum* (BJFC-S1951) **A, B** habit of conidiomata on branch **C** transverse section of conidioma **D** longitudinal section through conidioma **E, F** conidiogenous cells and conidia **G** top (left) and bottom (right) sides of colonies on potato dextrose agar (PDA) **H, I** conidia. Scale bars: 1000 µm (**A**); 200 µm (**B–D**); 10 µm (**E–F, H–I**).

Culture characters. Colonies on PDA with aerial mycelium gray-green, thick and dense, fluffly, margin with undulate and irregular, reverse with inky blue pigment accumulation, reaching 60 mm diam in 7 days at 25 °C.

Other material examined. CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, on the dead branches of *Fraxinus chinensis*, 19 April 2023, C.M. Tian, C. Peng, R. Yuan, M.W. Zhang & Y.Y. Wu, BJFC-S2366, living culture CFCC 70757; CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, on the dead branches of *Lagerstroemia indica*, 19 April 2023, C.M. Tian, C. Peng, R. Yuan, M.W. Zhang & Y.Y. Wu, BJFC-S1952, living culture CFCC 70758; *ibid*. BJFC-S2367, living culture CFCC 70759.

Notes. *Dothiorella hortiarborum* formed an independent clade with 87% MP, 97% ML, and 0.99 BYPP values and is distinct from *Do. acericola* and *Do. plurivora* in the multi-locus analyses (Fig. 3). Morphologically, *Do. hortiarborum* can be distinguished from *Do. acericola* by shorter conidia (Phookamsak et al. 2019) and *Do. plurivora* by smaller conidia (10.0–19.0 × 6.0–11.0 µm vs. 22.3–22.7 × 10.8–11.2 µm) (Abdollahzadeh et al. 2014). Additionally, *Do. hortiarborum* differs from *Do. acericola* in *tef1-α* (five bp difference from 170 characters, with 97.1% similarity, including no gaps) sequences, and *Do. plurivora* in *tef1-α* (one bp difference from 254 characters, with 99.6% similarity, including one gap), *tub2* (three bp difference from 370 characters, with 99.2% similarity, including one gap) sequences.

Phaeobotryon fraxini **Y.Y. Wu & C.M. Tian, sp. nov.**

MycoBank No: 851827

Fig. 6

Etymology. Named after the host, *Fraxinus chinensis*.

Holotype. CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, on the dead branches of *Fraxinus chinensis*, 19 April 2023, C.M. Tian, C. Peng, R. Yuan, M.W. Zhang & Y.Y. Wu (holotype BJFC-S1953, ex-type cultures CFCC 70762).

Description. ***Sexual morph*****:** Not observed. ***Asexual morph*****: *Conidiomata*** pycnidial, scattered, occasionally aggregated, superficial or immersed, globose, dark brown to black, unilocular, 200–360 µm diam. ***Disc*** inconspicuous. ***Ostioles*** single, brown or black, circular, central, papillate, 40–85 µm diam. ***Locules*** single, globose, 100–170 µm, ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** hyaline, smooth, thin-walled, holoblastic, cylindrical, formed from the cells lining the inner walls of the locules, 7.0–14.0 × 1.0–5.0 µm (av. ± S.D.= 10.6 ± 2.0 × 3.1 ± 0.8 µm). ***Conidia*** initially hyaline, smooth, thin-walled, then gradually producing light yellow pigment, becoming yellow or light brown, occasionally with bubbles, mature with 1-septate, brownish yellow to dark brown, oblong, obtuse, rounded at both ends, 13.0–20.0 × 7.0–10.0 µm (av. ± S.D.= 17.6 ± 1.3 × 8.7 ± 0.7 µm).

Culture characters. Colonies on PDA with aerial gray-white mycelium, thick and dark black at the edge, thin and paler in color in the center, fluffly, entire margin, reverse with black pigment accumulation, reaching 60 mm diam in 7 days at 25 °C.

Other material examined. CHINA, Beijing City, Tongzhou District, Central Green Forest Park, 39°52'16"N, 116°42'04"E, on the dead branches of *Fraxinus chinensis*, 19 April 2023, C.M. Tian, C. Peng, R. Yuan, M.W. Zhang & Y.Y. Wu, BJFC-S2368, living culture CFCC 70763.

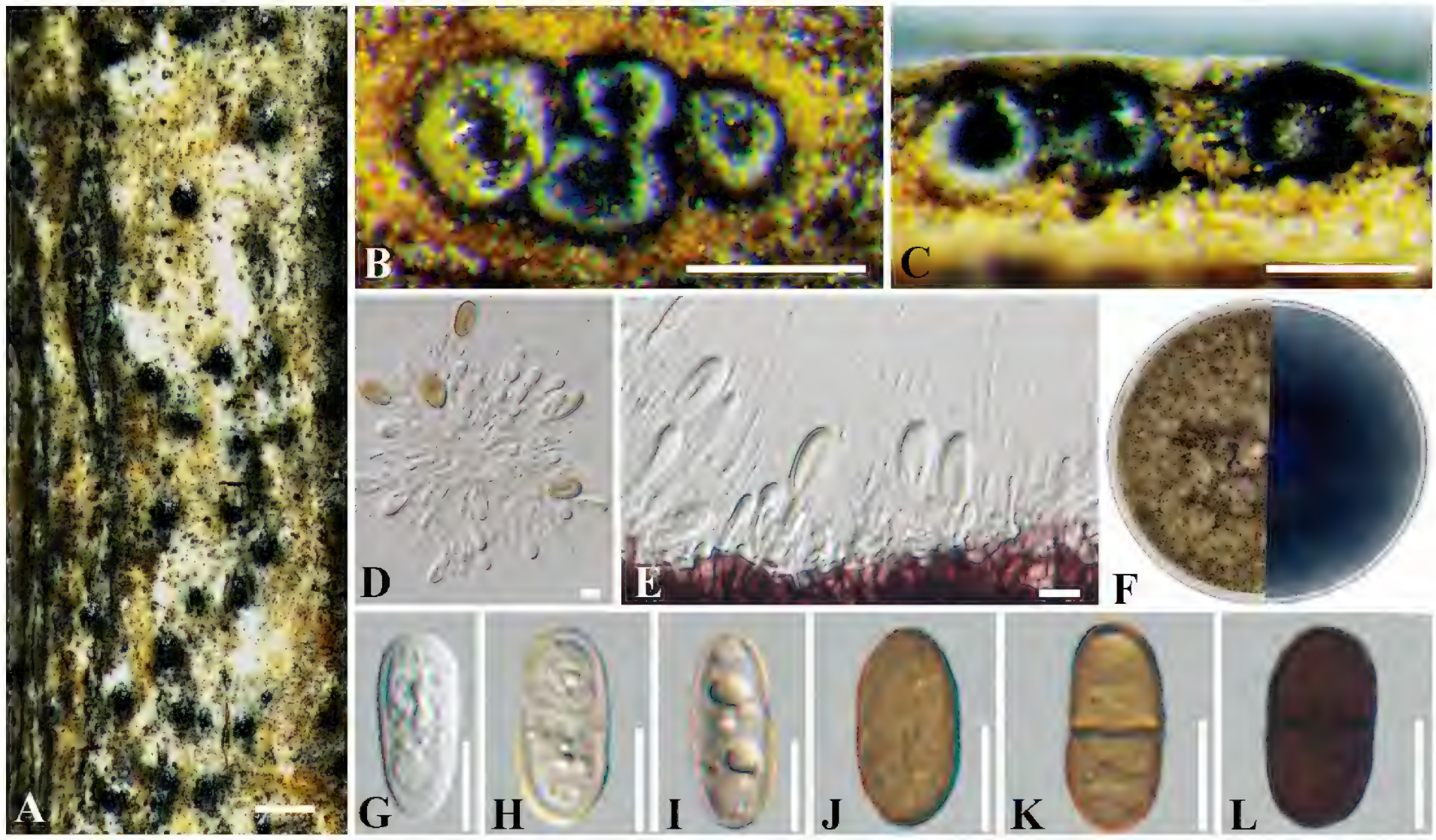

Figure 6. *Phaeobotryon fraxini* (BJFC-S1953) **A** habit of conidiomata on branch **B** transverse section of conidioma **C** longitudinal section through conidioma **D, E** conidiogenous cells and conidia **F** top (left) and bottom (right) sides of colonies on potato dextrose agar (PDA) **G-L** conidia. Scale bars: 500 μm (**A**); 200 μm (**B, C**); 10 μm (**D, E, G–L**).

Notes. Based on multi-locus phylogenetic analysis, the two isolates cluster separately in a high-supported clade with 100% MP, 100% ML, and 1.00 BYPP value (Fig. 4). In the phylogenetic analysis, *Phaeobotryon fraxini* showed a close relationship to *P. mali* and *P. rhois*. These three species could be distinguished based on ITS, *tef1-α*, and LSU loci from *P. mali* by nineteen bp (6/465 in ITS; 10/184 in *tef1-α*; 3/559 in LSU) and *P. rhois* by twenty-two bp (7/465 in ITS; 12/184 in *tef1-α*; 3/559 in LSU). Moreover, *P. fraxini* differs from *P. mali* and *P. rhois* in having smaller conidia (13.0–20.0 × 7.0–10.0 μm vs. 22.0–31.5 × 12–16.5 μm for *P. mali* and 20–25 × 10–12 μm for *P. rhois*) (Fan et al. 2015b; Jia et al. 2023) (Table 3). Therefore, *P. fraxini* is introduced as a novel species.

Table 3. Comparison of species in *Phaeobotryon*.

Species	Host	Location	Conidial size	Septation	Reference
Phaeobotryon aplosporum	*Rhus typhina*	China	17–19 × 5.5–7	aseptate	Pan et al. 2019
P. mali	*Malus pumila*	China	22.0–31.5 × 12–16.5	1-septate	Jia et al. 2023
P. cupressi	*Cupressus sempervirens*	Iran	24.1–25 × 12.2–12.5	1(–2)-septate	Abdollahzadeh et al. 2009
P. fraxini	*Fraxinus chinensis*	China	13–20 × 7–10	1-septate	This study
P. juniperi	*Juniperus formosana*	China	24.5–27.5 × 12.0–13.5	1-septate	Peng et al. 2023
P. mamane	*Sophora chrysophylla*	USA	35–38 × 14–15	1(–2)-septate	Phillips et al. 2008
P. negundinis	*Acer negundo*	Russia	16–24.5 × 7.9–11.5	aseptate	Daranagama et al. 2016
P. platycladi	*Platycladus orientalis*	China	23.0–31.0 × 9.5–12.5	aseptate or 1-septate	Lin et al. 2023a
P. rhoinum	*Rhus typhina*	China	19–21 × 7.5–9	1-septate	Zhu et al. 2018
P. rhois	*Rhus typhina*	China	20–25 × 10–12	1-septate	Fan et al. 2015b
P. spiraeae	*Spiraea salicifolia*	China	23.5–28.5 × 8.5–13.5	aseptate	Jin and Karunarathna 2021
P. ulmi	*Ulmus laevis*	Germany	28.5–32.5 × 16.5–18.5	aseptate or 1-septate	Zhang et al. 2021

Discussion

In this paper, 23 Botryosphaeriales isolates were identified as six species based on multi-locus phylogenetic analyses. These species included two new species, namely *Dothiorella hortiarborum* and *Phaeobotryon fraxini*, and four new hosts: *Aplosporella ginkgonis* on *Cotinus coggygria* var. *cinereus*; *A. javeedii* on *Acer miyabei*; *Acer truncatum*; *Forsythia suspensa*; *Lagerstroemia indica*; *Sambucus williamsii*; *Syringa vulgaris*; *Ulmus pumila*; *Xanthoceras sorbifolium*; *A. yanqingensis* on *Acer truncatum*; and *Do. acericola* on *Forsythia suspensa*; *Ginkgo biloba*; and *Syringa oblata*. The six fungal species identified in this study involve a total of 13 different hosts, which elucidates the wide range of hosts of Botryospaeriales.

Aplosporella is the type genus of Aplosporellaceae (Slippers et al. 2013). The distinctive morphological feature of *Aplosporella* species is that both ascospores and conidia are aseptately hyaline to pigmented (Slippers et al. 2013; Phillips et al. 2019). In this study, a total of three new host record species of the genus were identified, including *A. ginkgonis*, *A. javeedii*, and *A. yanqingensis*. *Aplosporella javeedii* has the highest isolation rate and the widest host range, involving five orders of host plants, including Dipsacales, Fabales, Lamiales, Myrtales, and Rosales. Currently, this species is mainly found in warm temperate and tropical regions (Fan et al. 2015a; Zhu et al. 2018), and further exploration is needed to determine whether the geographic range of *A. javeedii* is related to climate.

Dothiorella was considered a synonym of *Diplodia* based on a broad morphological concept (Crous and Palm 1999). Phillips et al. (2005) compared the morphological characteristics again and found that the conidia of *Dothiorella* were brown, with 1-septate in early development, and the conidia still adhered to the conidiogenous cells. In contrast, the conidia of *Diplodia* become black and septate after being excreted from the conidiomata. Crous et al. (2006) confirmed these morphological differences. Therefore, *Dothiorella* is regarded as an independent genus in the Botryosphaeriaceae. In this study, the conidia of *Do. hortiarborum* are transparent and aseptate when attached to conidiogenous cells. After being released by the conidiomata, the conidia bear yellowish pigment or become brown with a 1-septate. In recent years, many new species of *Dothiorella* have been published with conidial morphology similar to *Do. hortiarborum* (Li et al. 2023; Lin et al. 2023a; Wu et al. 2023). These suggest that the morphological characteristics of *Dothiorella* are not always stable. Thus, it is not accurate to rely solely on the morphology of conidia for *Dothiorella*; combining phylogenetic analysis and the size of conidia of neighboring species is necessary. *Dothiorella* species have been reported on more than 20 host plants in China (https://fungi.ars.usda.gov/). This study has expanded its host range in Oleaceae plants (*Do. acericola* in *Forsythia suspensa*, *Ginkgo biloba* and *S. oblata*, and *Do. hortiarborum* in *Fraxinus chinensis*).

Currently, many *Dothiorella* species have been recorded from *Fraxinus*, distributed mainly in regions such as Europe and North America (Table 4). In this study, a new species, *Do. hortiarborum*, from *F. chinensis*, was introduced in China. However, based on morphological and DNA sequence data, *Do. hortiarborum* shows significant differences from other species in *Fraxinus*. Phylogenetic analysis showed that *Do. hortiarborum* belongs to a different lineage from *Do. omnivora*, *Do.* sp.,

Table 4. Comparison of species from *Fraxinus* in *Dothiorella*.

Specise	Host	Location	Conidial size	Septation	Reference
Dothiorella concaviuscula	*Fraxinus viridis*	USA	4–6 × 2.5–3	no description	Jepson 1896
Do. fraxini	*Fraxinus* sp.	Belgium	26–30 × 12	1-septate	Saccardo 1892
Do. fraxinicola	*Fraxinus* sp.	USA	18–30 × 6–7	no description	Ellis and Everhart 1895
Do. hortiarborum	*Fraxinus chinensis*	China	10.0–19.0 × 6.0–11.0	1-septate	This study
Do. omnivora	*Fraxinus excelsior*	Bosnia	19.3–25.5 × 7.5–10.6	1-septate	Linaldeddu et al. 2016
Do. sp.	*Fraxinus excelsior*	Bosnia, Herzegovina	11–14 × 6–8	2–4-septate	Zlatković et al. 2016
Do. vidmadera	*Fraxinus ornus*	Australia	21.2–21.9 × 9.6–9.8	1-septate	Pitt et al. 2013

and *Do. vidmadera* (Fig. 3), while distinguishing them based on the size of conidia and the number of septates (Table 4). *Do. concaviuscula*, *Do. fraxini*, and *Do. fraxinicola* were not available for sequence information due to their earlier publication; however, *Do. hortiarborum* can also be easily distinguished from them based on their documented conidia size. In addition, *Do. lagerstroemiae* and *Do. hortiarborum* were both isolated from *Lagersiroemia alba*, but its conidia were significantly smaller than *Do. hortiarborum* (8.3–10 × 3.5–4 µm vs. 10.0–19.0 × 6.0–11.0 µm).

Phaeobotryon species have more overlapping morphological characters, with 1(–2) septate or aseptate conidia and similar pigmentation variations. For example, *P. cupressi* and *P. juniperi* have overlapping sizes of conidia (24.1–25 × 12.2–12.5 µm vs. 24.5–27.5 × 12.0–13.5 µm), *P. rhoinum* and *P. rhois* are derived from the same host and geographic origin, and the conidia have 1-septate (Table 3). So, morphology combined with phylogenetics to further clarify the affinities between species is essential. Furthermore, Phaeobotryon species were reported on a variety of hosts and considered to be potential or opportunistic pathogens (Weiland et al. 2016; Zhu et al. 2020; Ilyukhin and Ellouze 2023; Jia et al. 2023). In this study, *P. fraxini* was isolated only from dead *Fraxinus chinensis*; more extensive specimen collection was needed to confirm its distribution characteristics and pathogenicity.

Although Botryosphaeriales recorded many fungi on Index Fungorum (https://www.indexfungorum.org/), only some species are now recognized. Mainly due to the early records of many species, the lack of model specimens, or the low quality of specimens, it is difficult to obtain strains and DNA data. Therefore, more detailed sampling is needed to revise the classification system of related taxa in Botryosphaeriales.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization, Yingying Wu and Chengming Tian; data curation, Yingying Wu; funding acquisition, Chengming Tian; investigation, Yingying Wu, Cheng Peng, Rong Yuan, Mingwei Zhang, Yang Hu; project administration, Chengming Tian; resources, Yingying Wu, Cheng Peng, Rong Yuan, Mingwei Zhang, Yang Hu; supervision, Chengming Tian; writing-original draft, Yingying Wu; writing-review and editing, Yingying Wu, Cheng Peng, and Chengming Tian. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Aplosporella

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Supplementary material 2

Dothiorella

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Supplementary material 3

Phaeobotryon

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